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Multimodal Characterization of Proliferative Diabetic Retinopathy Reveals Alterations in Outer Retinal Function and Structure

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Abstract

Purpose—To identify changes in retinal function and structure in persons with proliferative diabetic retinopathy (PDR), including the effects of panretinal photocoagulation (PRP).

Design—Cross-sectional study

Participants—30 adults who received PRP for PDR, 15 adults with untreated PDR, and 15 age-matched controls

Methods—Contrast sensitivity, frequency doubling perimetry (FDP), Humphrey visual fields, photostress recovery, and dark adaptation were assessed in all subjects. Fundus photography and macular spectral-domain optical coherence tomography (SD-OCT) were also obtained. SD-OCT scans were semi-automatically segmented to quantify retinal layer thicknesses.

Main Outcome Measures—Visual function test results were compared between patients with PDR and PRP, untreated patients with PDR, and controls. Mean retinal layer thicknesses were also compared between groups. Correlation analyses were performed to evaluate associations between visual function test results and retinal layer thicknesses.

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This article contains additional online-only material. Supplemental materials are provided at the end of the online version of this manuscript. The following should appear online-only: Tables 3, 5, 6, 7, 8, and 9.

Results—Patients with PDR exhibited significant reduction of FDP mean deviation (MD) in PRP-treated ($MD \pm SD: -8.20 \pm 5.76$ dB, $p < 0.0001$) and untreated (-5.48 ± 4.48 dB, $p < 0.0001$) patients relative to controls (1.07 ± 2.50 dB). Reduced log contrast sensitivity compared with controls (1.80 ± 0.14) was also observed in both PRP-treated (1.42 ± 0.17 , $p < 0.0001$) and untreated (1.56 ± 0.20 , $p = 0.001$) patients with PDR. Compared to controls, patients treated with PRP demonstrated increased photostress recovery time (151.02 ± 104.43 sec vs 70.64 ± 47.14 sec, $p = 0.001$) and dark adaptation speed (12.80 ± 5.15 min vs 9.74 ± 2.56 min, $p = 0.022$) whereas untreated patients had no significant differences in photostress recovery time or dark adaptation speed relative to controls. PRP-treated patients had diffusely thickened nerve fiber layers ($p = 0.024$) and diffusely thinned retinal pigment epithelial layers (RPE) ($p = 0.009$) versus controls. Untreated patients with PDR also had diffusely thinned RPE layers ($p = 0.031$) compared to controls.

Conclusions—Patients with untreated PDR exhibit inner retinal dysfunction, as evidenced by reduced contrast sensitivity and FDP performance, accompanied by alterations in inner and outer retinal structure. PRP-treated patients had more profound changes in outer retinal structure and function. Distinguishing the effects of PDR and PRP may guide the development of restorative vision therapies for patients with advanced diabetic retinopathy.

INTRODUCTION

The International Diabetes Federation estimated that the prevalence of diabetes in 2013 was 382 million people worldwide, and it is expected to reach 592 million people by 2035.¹ Diabetic retinopathy affects approximately 35% of persons with diabetes and PDR affects approximately 7% of persons with diabetic retinopathy.² Therefore, PDR and its consequences continue to be a major public health challenge.

Meyer-Schwickerath developed retinal laser photocoagulation for the treatment of proliferative diabetic retinopathy (PDR) in the 1950s, and panretinal photocoagulation (PRP) remains the most widespread treatment for PDR nearly 60 years later.³ PRP induces regression of neovascularization within several weeks of treatment, presumably due to reduction of metabolic demand.⁴ It has traditionally been assumed that PRP kills poorly perfused cells in the neurosensory retina, the retinal pigment epithelium (RPE) and the photoreceptor layers of the peripheral retina, reducing angiogenic signaling and oxidative stress.

However successful at preventing blindness, PRP invariably causes retinal damage and unwanted visual side effects, including constricted visual fields, reduced visual acuity, altered color vision, impaired dark adaptation, and decreased contrast sensitivity.⁵⁻¹¹ PRP also compromises retinal structure, with thinning of the nerve fiber layer, focal retinochoroidal atrophy at burn locations, and scar formation with progressive expansion.¹²⁻¹⁶ Thus, PRP superimposes thermal injury-induced retinal degeneration onto the intrinsic neurodegeneration of diabetic retinopathy, leaving patients with reduced abilities to drive and read, particularly under low light conditions.¹⁷

The cellular mechanisms by which persons with PDR lose vision remain unclear, so this study was conducted to test the hypothesis that PRP induces outer retinal dysfunction in patients with PDR.

By evaluating retinal structure and function within the same patients, this study additionally aimed to correlate changes in retinal structure with specific visual deficits in PDR. Improved understanding of the pathogenesis of visual dysfunction in individuals with PDR and in those who have received PRP could lead to the identification of therapeutic targets for these patients.

MATERIALS AND METHODS

This study was conducted at the University of Michigan W. K. Kellogg Eye Center after approval by the University of Michigan Medical School Institutional Review Board. Participants were recruited from the clinics and through the University of Michigan Clinical Studies website from August 2012 through October 2013. Informed consent was obtained from all subjects prior to participation in the study. This research adhered to the tenets of the Declaration of Helsinki and the Health Insurance Portability and Accountability Act.

PATIENT ENROLLMENT AND BASELINE EVALUATION

Three groups of patients were enrolled: adults with type 1 or type 2 diabetes who had undergone PRP for PDR (post-PRP group), adults with type 1 or type 2 diabetes with PDR and no history of PRP (treatment-naïve group), and healthy adults (control group).

Inclusion criteria for the post-PRP group were: (1) diabetes mellitus as defined by the American Diabetes Association diagnostic criteria¹⁸; (2) age ≥ 18 years old; (3) best corrected visual acuity (BCVA) of 20/400 or better in the study eye; and (4) PRP administered ≥ 6 months prior to enrollment. Inclusion criteria for the treatment naïve group were: (1) diabetes mellitus; (2) age ≥ 18 years old; (3) BCVA of 20/400 or better in study eye; and (4) evidence of active PDR on dilated fundus exam or fundus photography. Inclusion criteria for the control group were: (1) age ≥ 18 years old; (2) BCVA of 20/30 or better in study eye, and (3) no diabetes mellitus.

Exclusion criteria for the post-PRP group and treatment-naïve groups were: (1) any eye disease other than PDR; (2) history of drug or alcohol abuse; (3) neurologic or systemic disease that could impair vision; (4) hospitalization within 1 month prior to enrollment; (5) difference in two recent consecutive hemoglobin A_{1c} measurements ≥ 5%; (6) unable to give informed consent or unable to complete testing; (7) spherical equivalent > ± 6.00 diopters; (8) pregnant or nursing; (9) blood pressure ≥ 180/100 mmHg. Exclusion criteria for control subjects were: (1) spherical equivalent > ± 6.00 diopters; (2) pregnant or nursing; (3) ocular, neurologic, or systemic disease that could impair vision; and (4) unable to give informed consent or unable to complete testing.

One study eye was chosen in each patient and if both eyes met the eligibility criteria, the eye with the better visual acuity was examined. All subjects underwent ophthalmologic examination including refraction and measurement of BCVA using the electronic visual

acuity tester (EVA) with E-ETDRS protocol, applanation tonometry, slit-lamp examination, and dilated fundus exam. A blood sample was obtained from each participant to measure HbA_{1c}.

Following refraction and measurement of best-corrected visual acuity, all patients underwent a series of functional visual tests in their study eye: contrast sensitivity, frequency doubling perimetry (FDP), Humphrey visual fields, color vision, Minnesota reading acuity, photostress recovery, and dark adaptation. Contrast sensitivity was measured monocularly using the Pelli-Robson contrast sensitivity chart (Haag-Streit USA, Mason, Ohio, USA), read at 1 meter under standard overhead lighting conditions. Patients were asked to read through each line on the chart until two or three letters in a triplet were read incorrectly. The logarithmic contrast sensitivity value of the previous triplet of letters determined the patient's contrast sensitivity score. FDP was performed with the 24-2 full threshold testing strategy using the Matrix perimeter (Carl Zeiss Meditec, Dublin, California, USA) as described by Jackson et al.¹⁹ Photopic central 10-2 SITA standard and peripheral 60-4 threshold visual fields were determined with a Humphrey Field Analyzer (Humphrey Field Analyzer, II-750; Carl Zeiss Meditec, Dublin, CA). For both FDP and standard Humphrey perimetry the reliability criteria used were < 33% fixation errors, < 33% false-positive errors, and < 33% false negative errors. The Farnsworth D15 test was administered to all study participants and a fail on the Farnsworth D15 was defined as two or more diametrical crossings on the test. The test was performed monocularly under a Macbeth lamp providing 270 lux illumination. The Minnesota Reading test (MNREAD) was administered to assess reading acuity. The MNREAD acuity chart contains continuous text phrases in 19 different font sizes, and subjects are timed as they read progressively smaller lines of text. Photostress recovery time was determined by exposing the undilated study eye to a penlight (5,000-8,000 lux) for 45 seconds and measuring the time until the subject could read one ETDRS line above his/her best corrected visual acuity. Dark adaptation speed was assessed using the AdaptDx dark adaptometer (MacuLogix, Hummelstown, Pennsylvania) that measures the sensitivity and recovery of rod photoreceptors after bleaching with a 5.8×10^4 scotopic cd/m² sec flash.¹⁹ The rod intercept value was used to characterize dark adaptation speed. This method provides information about the area of the retina that was not damaged by PRP.

Fundus photography and spectral domain optical coherence tomography (SD-OCT) were performed on all study eyes. A 135 degree two-wavelength scanning laser ophthalmoscope (SLO) image centered on the macula was taken of each study eye using the Optos camera (Optos®, Dunfermline, United Kingdom). Diabetic retinopathy was graded as no DR, mild or moderate nonproliferative DR (NPDR), or PDR by two independent graders. Additionally, a 20° by 20° SD-OCT cube scan (97 sections, 512 A-scans in each B-scan, and 3.87 micron axial resolution) of the macula of the dilated study eye was obtained with a Spectralis SD-OCT (Heidelberg Engineering, Heidelberg, Germany). Retinal layer thicknesses in macular cube scans were analyzed in 9 ETDRS areas: a 1 mm central circle at the fovea, surrounded by a 3 mm inner ring and a 6 mm outer ring. Both the 3 mm and 6 mm rings were further sectioned into superior, nasal, inferior, and temporal quadrants. In each retinal B-scan, we measured the thicknesses of the nerve fiber layer (NFL), ganglion cell layer + inner plexiform layer (GCL+IPL), inner nuclear layer (INL), outer plexiform layer

and outer nuclear layer (OPL+ONL), inner segment/outer segment photoreceptor layer (IS/OS), and retinal pigment epithelium (RPE). The upper and lower boundaries defining the thicknesses of these seven layers are defined in our previous publication.²⁰

Segmentation of retina layer boundaries on each SD-OCT retinal B-scan was performed semi-automatically in two steps. First, we utilized automatic layer segmentation software, Duke Optical Coherence Tomography Retinal Analysis Program (DOCTRAP), to attain the eight retinal boundaries on each B-scan.^{20, 21} In the second step, all segmented eight retinal boundaries for all B-scans were carefully monitored by an expert manual grader and the boundary lines were adjusted by the grader utilizing DOCTRAP's graphical user interface. A second expert grader separately reviewed the first grader's markings for possible mistakes in marking. One low-quality SD-OCT scan from one patient was excluded from the analysis due to an inability to obtain a consistent and reproducible segmentation.

STATISTICAL ANALYSIS

Data were double-entered and verified for accuracy and analysis was performed using SPSS Statistics (version 21.0; SPSS, Inc, Chicago, IL, USA) and SAS, Version 9.3.0 (SAS Institute, Cary, North Carolina). Baseline data for demographic variables are presented by mean and standard deviation. The Shapiro-Wilk test was used to check for normal distribution of data for each variable. Categorical variables were compared between groups by chi-squared or Fischer's exact tests. Independent t-tests and Mann-Whitney U tests were used to compare continuous variables between groups. For variables that were not normally distributed, the nonparametric analysis of covariance model developed by Schacht et al. was used for comparisons between Post-PRP PDR and Treatment-naïve PDR groups, adjusting for age as a covariate.²² For comparing continuous variables among all three groups, analysis of variance (ANOVA) or Kruskal-Wallis tests were performed as appropriate.

The mean values for retinal layer thicknesses in each patient group were compared with Kruskal-Wallis tests. When there were significant differences in layer thickness between the three groups, Mann-Whitney tests were performed to compare layer thicknesses between the 3 patient groups. An analysis of covariance (ANCOVA), controlling for age as a covariate, was also used to compare global retinal layer thicknesses between study groups. Spearman's correlation test was used to investigate associations between variables. Statistical significance was defined as a p-value < .05.

RESULTS

Baseline Demographics and Visual Acuity

This study included 60 eyes from 60 age-matched volunteers, including 30 diabetic patients who had undergone PRP for PDR (post-PRP group), 15 diabetic patients with PDR who had not yet undergone PRP (treatment-naïve group), and 15 healthy adults (control group) (Table 1). Patients in the post-PRP cohort were seen at an average of 10.9 ± 10.3 years after treatment, with a range of 1 to 38 years post-treatment. Mean visual acuity was significantly reduced in both post-PRP ($p < 0.0001$) and treatment-naïve patients ($p = 0.001$) compared to controls. Both groups of patients with PDR demonstrated a mean visual acuity reduction

>10 letters compared to controls in spite of the absence of macular edema. The post-PRP group had a longer average duration of diabetes, but a lower mean HbA_{1c} than the treatment-naïve group.

Visual Function Analysis

Previous studies have examined various components of retinal structure and function in persons with PDR; this study is distinctive in that it evaluated multiple aspects of visual function to gain a comprehensive assessment of the impact of diabetes and its treatment (Table 2). Functions of the macula were evaluated in five ways. First, contrast sensitivity testing showed that both treated ($p<0.0001$) and untreated ($p=0.001$) patients with PDR exhibited reduced contrast sensitivity compared to controls. Seventy-three percent of treated patients and 53% of untreated patients with PDR demonstrated contrast sensitivity below the normal reference range (Figure 1). PRP-treated patients had significantly lower log contrast sensitivity than untreated patients ($p=0.024$). This difference remained significant when age was controlled for ($p=0.032$). Reading acuity, which measures the smallest font persons can read accurately, was reduced by > 2 lines in treated ($p<0.0001$) and greater than one line in untreated ($p<0.0001$) patients with PDR compared to controls.

Secondly, Matrix FDP testing also revealed significant differences between groups. Compared to controls, both treated ($p>0.0001$) and untreated ($p=0.001$) patients had < 7 dB reduction in sensitivity to the central foveal target. The entire Matrix FDP field, measured by the mean deviation (MD), was also depressed on average by 9.3 dB in the post-PRP group ($p<0.0001$) and by 6.6 dB in the treatment-naïve group ($p<0.0001$) compared to controls, whereas FDP MD and foveal sensitivity were equivalent between treated and untreated PDR patients. Compared to controls, both treated ($p<0.0001$) and untreated ($p<0.0001$) patients with PDR had increased variability across the FDP pattern as shown by the pattern standard deviation (PSD), and post-PRP patients had greater PSD values compared to untreated patients ($p=0.003$).

Third, central light-adapted visual fields, as evaluated by the Humphrey Field Analyzer 10-2 SITA standard mean deviation, were depressed by on average 4.6 dB in the post-PRP group ($p<0.0001$) and by 3.3 dB in the treatment-naïve ($p<0.0001$) group compared to controls. HFA PSD was also increased in both treated ($p<0.0001$) and untreated ($p<0.0001$) patients with PDR compared to controls. There were no differences in HFA 10-2 MD or PSD between treated and untreated patients with PDR.

Fourth, photostress testing showed that patients in the post-PRP group had significant increases in photostress recovery time ($p=0.001$) and dark adaptation speed ($p=0.022$) relative to controls. On average, photostress recovery time was prolonged by 80 seconds and dark adaptation speed was prolonged by roughly 3 minutes. However, there was substantial variability among post-PRP patients in photostress recovery time and dark adaptation speed, even between patients with similar visual acuities and similarly dense laser lesions (Figure 2). Patients in the post-PRP group tended to have prolonged photostress recovery times ($p=0.060$, and $p=0.093$ when controlling for age) compared to the treatment-naïve subjects. Overall, the treatment-naïve group did not have significantly prolonged mean photostress recovery time or dark adaptation speed compared to controls.

Fifth, color sensitivity, as assessed by the Farnsworth D15, was impaired in 9/30 patients treated for PDR, 4/15 untreated patients with PDR, and 2/15 controls. Tritan defects were identified in 23.3% of post-PRP patients, 26.7% of treatment-naïve patients, but in no control patients.

Taken together, these results reveal marked impairment of the inner retina, as revealed by contrast sensitivity and FDP, as well as cone photoreceptors, as assessed by 10-2 fields, photostress responses and color vision testing among patients with PDR relative to controls.

In addition, peripheral visual fields were assessed by the Humphrey Field Analyzer 60-4 test. The threshold visual sensitivities of the nasal, temporal, superior, and inferior zones were combined to establish the total 4-quadrant threshold. The total 60-4 threshold was reduced in both post-PRP ($p<0.0001$) and treatment-naïve patients ($p<0.0001$) compared to controls.

Ninety-seven percent of post-PRP patients and 67% of treatment-naïve patients scored below the reference range (Figure 1). Treated patients with PDR had significantly lower 4-quadrant thresholds than untreated patients ($p<0.0001$, and $p<0.0001$ when controlling for age).

Further analysis of the visual function data (Figure 1) shows that untreated eyes with PDR most commonly had reduced sensitivity (MD) as measured by 10-2 and 60-4 HFA (73% and 67%, respectively) and FDP MD (67%), followed by reduced visual acuity (60%), contrast sensitivity and reading acuity (both 53%), FDP foveal threshold (40%), and photostress recovery (13%); none of the untreated eyes had prolonged dark adaptation times. After PRP, a higher percentage of patients exhibited impairment in all visual function parameters, with the exception of FDP foveal threshold, which was reduced in a lower proportion of post-PRP patients, and visual acuity, which was reduced in a comparable percentage of patients. The deterioration of peripheral retinal and macular function is largely predicted on the basis of prior reports whereas the apparent improvement in FDP foveal threshold is a novel and currently unexplained observation.^{5, 9, 23}

The comprehensive analysis of retinal function facilitates comparison of patients. For example, Figure 2 shows two-wavelength SLO images of two individuals with similar patterns of PRP scars and excellent visual acuities but widely different responses on FDP, photostress, and dark adaptation testing. These findings show that fundus appearance alone does not predict visual function.

To investigate for associations between blood glucose control or duration of diabetes and visual function test results, the post-PRP and treatment-naïve groups were combined and HbA_{1c} and duration of diabetes were correlated with each of the visual function test results. Higher HbA_{1c} values correlated with faster dark adaptation speeds (Spearman $r=0.338$, $p=0.033$) and improved Humphrey 60-4 visual field total thresholds (Spearman $r=0.334$, $p=0.027$). These associations were not significant when the analysis controlled for history of PRP, suggesting that these relationships were spurious and that the differences were due to better diabetes control in post-PRP patients than in treatment-naïve patients. No other visual function tests were related to HbA_{1c} levels. Longer duration of diabetes was associated with

lower Humphrey 60-4 total thresholds (Spearman $r = 0.586$, $p < 0.0001$). This relationship remained significant after controlling for history of PRP. No other visual function tests were related to duration of diabetes.

Retinal Structure Analysis and Association with Visual Function

PDR and subsequent PRP both lead to substantial alterations in retinal structure.¹⁴⁻¹⁶ Figure 3 and Table 3 (available at www.aaojournal.org) show the mean macular nerve fiber layer thickness was significantly greater in post-PRP patients compared to controls in the temporal outer quadrant ($p < 0.0001$), superior outer quadrant ($p < 0.0001$), nasal outer quadrant ($p = 0.002$), inferior outer quadrant ($p = 0.001$), temporal inner quadrant ($p < 0.0001$), superior inner quadrant ($p = 0.002$), nasal inner quadrant ($p < 0.0001$), and inferior inner quadrant ($p = 0.001$). Post-PRP patients also had thicker nerve fiber layers than treatment-naïve patients in the temporal outer quadrant ($p = 0.022$), nasal outer quadrant ($p = 0.034$), nasal inner quadrant ($p = 0.011$), and inferior inner quadrant ($p = 0.023$). As displayed in Table 4, global nerve fiber layer thickness was thicker in Post-PRP PDR patients than in treatment-naïve PDR patients ($p = 0.026$) and this difference remained significant when controlling for age ($p = 0.024$). Treatment-naïve patients also trended towards thicker nerve fiber layers than controls, but this trend was only significant in the temporal outer quadrant ($p = 0.009$) and temporal inner quadrant ($p = 0.021$).

Post-PRP patients had increased global NFL thickness associated with a greater number of years since receiving treatment (Spearman $r = 0.444$, $p = 0.026$). Among all patient groups, increased global NFL thickness was associated with reduced FDP MD (Spearman $r = 0.544$, $p < 0.0001$) and reduced contrast sensitivity (Spearman $r = 0.422$, $p = 0.001$). Increased global NFL thickness was also marginally correlated with longer photostress recovery times (Spearman $r = 0.266$, $p = 0.042$).

GCL+IPL thickness was decreased in post-PRP patients compared to controls in the temporal inner quadrant ($p = 0.028$), superior inner quadrant ($p = 0.004$), nasal inner quadrant ($p = 0.007$), and inferior inner quadrant ($p = 0.001$) regions. In the treatment-naïve PDR group, mean GCL+IPL thickness was decreased in the superior inner quadrant ($p = 0.036$), nasal inner quadrant ($p = 0.005$), and inferior inner quadrant ($p = 0.050$) compared to controls (Figure 3 and Table 5, available at www.aaojournal.org). Among all patient groups, increased GCL+IPL thickness was marginally associated with higher FDP mean deviation (Spearman $r = 0.311$, $p = 0.016$).

The mean INL thickness in the post-PRP group was reduced in the nasal outer quadrant ($p = 0.008$), superior inner quadrant ($p = 0.021$), and inferior inner quadrant ($p = 0.028$) and increased in the central field ($p = 0.012$) compared to controls. The INL central field was also thicker in post-PRP patients compared to treatment-naïve PDR patients ($p = 0.019$). Treatment-naïve patients demonstrated reduced INL thickness compared to controls in the nasal inner quadrant ($p = 0.016$) and inferior inner quadrant ($p = 0.016$) (Figure 3 and Table 6, available at www.aaojournal.org).

The OPL+ONL thickness was significantly greater in post-PRP patients compared to controls in the temporal outer quadrant ($p = 0.036$) and inferior outer quadrant ($p = 0.011$). In

treatment-naïve patients, the OPL+ONL had increased thickness in the temporal outer quadrant ($p<0.0001$), superior outer quadrant ($p=0.009$), inferior outer quadrant ($p=0.021$), temporal inner quadrant ($p=0.002$), and superior inner quadrant ($p=0.018$). The global OPL+ONL thickness was greater in treatment-naïve patients compared to controls ($p=0.003$) and this difference remained significant after controlling for age ($p=0.001$) (Table 4). The OPL+ONL was thicker in treatment-naïve patients than in treated patients in the temporal outer quadrant ($p=0.013$), superior outer quadrant ($p=0.011$), temporal inner quadrant ($p=0.047$), and superior inner quadrant ($p=0.041$) (Figure 3 and Table 7, available at www.aaojournal.org). The global OPL+ONL thickness was greater in treatment-naïve patients compared to treated patients ($p=0.033$) and this difference remained significant after controlling for age ($p=0.015$) (Table 4).

IS/OS layer thickness was reduced in post-PRP patients compared to controls in the temporal outer quadrant ($p=0.018$), superior outer quadrant ($p=0.017$), nasal outer quadrant ($p=0.028$), inferior outer quadrant ($p=0.003$), and central field ($p=0.006$). The global IS/OS thickness was reduced in post-PRP PDR patients compared to controls ($p=0.002$) and this difference remained significant after controlling for age ($p=0.011$). The IS/OS layer was also thinner in treatment-naïve patients compared to controls in the superior outer quadrant ($p=0.026$), inferior outer quadrant ($p=0.026$), and central field ($p=0.050$). Post-PRP patients trended towards thinner IS/OS layers than treatment-naïve patients, but there were no statistically significant differences in thickness between the two groups (Figure 3 and Table 8, available at www.aaojournal.org). Among all patient groups, reduced IS/OS global thickness was associated with reduced FDP mean deviation (Spearman's $r=0.438$, $p=0.001$) and reduced log contrast sensitivity (Spearman's $r=0.310$, $p=0.017$).

RPE thickness was reduced in post-PRP patients compared to controls in the temporal outer quadrant ($p=0.007$), superior outer quadrant ($p=0.021$), temporal inner quadrant ($p<0.0001$), superior inner quadrant ($p<0.0001$), nasal inner quadrant ($p<0.0001$), inferior inner quadrant ($p<0.0001$), and central field ($p=0.010$). Global RPE thickness was also reduced in post-PRP PDR patients compared to controls ($p=0.009$), and this relationship remained significant after controlling for age ($p=0.003$) (Table 4). In treatment-naïve PDR patients, thinning of the RPE was also observed in the temporal outer quadrant ($p=0.023$), temporal inner quadrant ($p=0.001$), superior inner quadrant ($p=0.015$), nasal inner quadrant ($p=0.008$), and inferior inner quadrant ($p=0.007$) compared to controls. Global RPE thickness was reduced in treatment-naïve PDR patients compared to controls ($p=0.031$), and this relationship remained significant after controlling for age ($p=0.013$) (Table 4). There were no significant differences in RPE thickness between treated and untreated PDR patients (Figure 3 and Table 9, available at www.aaojournal.org). Among all patient groups, reduced global RPE thickness was marginally associated with reduced FDP mean deviation (Spearman's $r=0.277$, $p=0.034$).

Taken together, these data reveal impairment of multiple aspects of visual function and marked structural alterations in both the inner and outer retinal layers in treated and untreated patients with PDR. Overall, post-PRP patients demonstrated greater visual impairment and more severe structural changes than treatment-naïve patients.

DISCUSSION

The purpose of this study was to quantify changes in multiple dimensions of retinal structure and function among individuals with untreated and treated PDR. By evaluating retinal structure and function within the same well-characterized cohort, this study also aimed to identify the changes occurring within specific retinal layers associated with visual dysfunction in PDR. Improved understanding of the pathogenesis of visual dysfunction in individuals with PDR and in those who have received PRP could lead to the identification of therapeutic targets for these patients. Collectively, the results reveal that PDR compromises the function of both inner and outer retinal neurons, and that of cone photoreceptors in particular after treatment with PRP, thus supporting the hypothesis.

In this study, inner retinal function was assessed by FDP and contrast sensitivity. The FDP response is thought to originate from ganglion cells of the magnocellular pathway.^{24, 25} All individuals with PDR, regardless of PRP treatment status, demonstrated impaired FDP performance in this study. These findings are unsurprising, given that previous studies have shown that FDP performance is depressed in persons with diabetes and mild or no retinopathy.^{19, 26} Parikh et al also evaluated FDP sensitivity in diabetic persons with advanced retinopathy and found a sensitivity of 90.5% and a specificity of 97.6% for very severe NPDR and untreated PDR.²⁷ These studies, in combination with our current findings, demonstrate that depressed FDP performance characterizes all stages of diabetic retinopathy, regardless of PRP treatment status.

Both treated and untreated patients with PDR had impaired contrast sensitivity that was significantly worse among patients who had received PRP. These findings align with previous demonstrations of impaired contrast sensitivity in diabetics with mild or no retinopathy, with greater impairment in individuals who received PRP for PDR or severe NPDR.^{5, 19, 28} The consistent demonstration of depressed contrast sensitivity and FDP performance in all stages of diabetic retinopathy further the hypothesis that dysfunction of inner retinal neurons is an early event in diabetic retinopathy that precedes PDR or its treatment with PRP¹⁷.

Inner retinal dysfunction in patients with PDR was accompanied by marked structural changes in the inner retina. This study observed thinning of the combined GCL and IPL in the pericentral macula in both untreated and treated patients with PDR compared to controls. Van Dijk et al previously demonstrated thinning of the GCL in the peripheral macula of diabetic individuals with no or minimal DR.²⁹ Thus, changes in inner retinal structure and reductions in inner retinal function occur in all stages of diabetic retinopathy.

Interestingly, this study observed marked and diffuse thickening of the NFL among patients treated with PRP. These data diverge from previous studies that have reported progressive thinning of the NFL following PRP.¹⁴⁻¹⁶ Two of these studies evaluated longitudinal changes in NFL thickness by following patients up to two years after PRP, and a third cross-sectional study evaluated patients at an average of 15 months after PRP treatment.¹⁴⁻¹⁶ Patients in our study were seen an average of 10.9 years after PRP, and the unexpected finding of thicker NFL in these patients may be explained by the later time point at which

they were examined. Indeed, a moderate association was found between NFL thickness and years since receiving PRP. The results of this study in combination with previous reports, suggest that NFL thickness can be increased or decreased in PRP-treated patients. Parallel findings have been reported in hereditary retinal degenerations, where OCT evaluations have demonstrated both thickening and thinning of the peripapillary NFL in patients with retinitis pigmentosa, choroideremia, and Stargardt disease.³⁰⁻³⁵

Further investigation is needed to understand the pattern and mechanism of NFL changes in PDR, but one possible explanation is glial cell proliferation within the NFL. Altered glial metabolism and glial reactivity have been implicated as important pathogenic events in diabetic retinopathy.³⁶⁻³⁸ Histologic studies on post-mortem eyes have substantiated evidence for glial reactivity in diabetes by demonstrating GFAP upregulation in the nerve fiber and ganglion cell layers, along with GFAP induction in the Müller cells of diabetic eyes. Additionally, Müller cells have been identified throughout the entire width of the retina in post-mortem eyes with PDR, suggesting that Müller cells migrate toward both the inner and outer retinal layers in diabetic retinopathy.^{39, 40} The histologic findings of glial activation and Müller cell migration in diabetic retinopathy suggests the hypothesis that the NFL thickening observed in this study reflects gliosis in the inner retina.

Peripheral retinal sensitivity, as assessed by 60-4 Humphrey visual fields, was reduced in all PDR patients, but to a greater degree in treated individuals. The finding that 67% of treatment-naïve patients had reduced peripheral sensitivity is supported by previous demonstrations of visual field defects in diabetic patients prior to PRP at sites of vascular compromise⁴¹. 10-2 Humphrey visual fields, reading acuity, and visual acuity were tested in all patients to assess cone function and macular integrity. All functional tests were impaired in both groups of patients with PDR, with no significant differences between PRP-treated and untreated individuals. However, the standard deviations for mean performance on these tests were large in both groups, indicating substantial within-group variability in visual function. Therefore, although this study detected no significant differences in central visual fields, reading acuity, or visual acuity between PRP-treated and untreated patients with PDR, it is difficult to interpret the impact of laser treatment given the wide variability in performance among the patients. These results parallel the findings of previous studies which have reported marked improvement or reduced visual acuity following PRP treatment.^{9, 23}

Visual acuity and central visual fields were similar in treated and untreated individuals with PDR, but visual function tests dependent on RPE-photoreceptor complex function distinguished post-PRP and treatment-naïve patients. For example, post-PRP PDR patients exhibited significantly longer dark adaptation times than controls. Dark adaptation times were also prolonged on average among treatment-naïve patients compared to controls, but the difference was not significant and not all treatment-naïve patients demonstrated impairment. The less pronounced kinetic abnormalities among the treatment-naïve PDR patients were surprising, given the results of previous studies. An advantage of measuring dark adaptation in the macula is that it evaluates the status of the region of the retina that was not damaged by the PRP, and might respond to therapeutic intervention, in contrast to

the midperipheral retina which is assessed by the Goldmann-Weekers device, and which would be unlikely to improve.

Critical review of the literature regarding dark adaptation in patients with PDR reveals marked disparities in testing methods (instruments used, bleaching stimulus intensity and location) and patient characteristics across studies. For example, Zetterstrom et al. recorded dark adaptation curves in diabetic patients with retinopathy before and after photocoagulation.⁴² They found that a majority of patients had impaired dark adaptation kinetics prior to PRP treatment, and that no appreciable worsening occurred following PRP. They used a Goldmann-Weekers dark adaptometer, which measures in the mid-peripheral retina and with a larger test target than the AdaptDx. By contrast, other studies reported no change in dark adaptation speed among individuals with early diabetic retinopathy, and other authors noted a wide range of dark adaptation speeds among diabetic patients with and without retinopathy^{43, 19}. Moreover, the patient characteristics are not well defined in several papers. Thus, the literature is surprisingly inconsistent regarding dark adaptation in PDR, and results of the present study unfortunately cannot be compared with those of other investigators. For that reason we are now preparing a separate manuscript that analyzes multiple features of dark adaptation in patients with a range of diabetic retinopathy severity. This in-depth analysis is beyond the scope of the current report.

There was a marginally significant depression ($p=0.06$) in photostress recovery time among PRP-treated patients compared to untreated patients. Additionally, post-PRP patients had markedly slower photostress recovery times than controls ($p=0.001$). Treatment-naïve patients trended towards longer photostress recovery times than controls, but the difference was not significant. Finally, color vision showed similar patterns of impairment in treated and untreated patients with PDR. Tritan (blue-yellow) defects were noted in 23.3% of post-PRP PDR patients, 26.7% of treatment-naïve PDR patients, and none of the controls. This clinical evidence for cone dysfunction in patients with PDR aligns well with previous histological studies that have shown selective S-cone losses in post-mortem diabetic eyes compared to controls.⁴⁴

Photostress testing uses intense light to bleach the visual pigments and induce temporary cone photoreceptor insensitivity. Recovery of normal photopic visual function depends on the anatomic integrity of the macula, transport of 11-*cis*-retinol from RPE and Müller cells to cones, and on synthesis of visual pigments by the RPE-photoreceptor complex.^{45, 46} Dark adaptation testing utilizes rapid bleaching of the retina, and time is measured until recovery of scotopic vision. This study observed similar visual acuity, reading acuity, and central visual field in treated and untreated patients with PDR, all of which rely on macular integrity. It seems likely, therefore, that the prolonged photostress recovery and dark adaptation times in post-PRP PDR patients reflect pathological changes in the RPE-photoreceptor complex rather than differences in integrity of the synaptic layers of the macula.

The SD-OCT results from this study also demonstrated thinned RPE layers in comparison to controls. Additionally, thinned IS/OS layers were observed in patients who had received PRP. The RPE plays a central role in supporting photoreceptor function by supplying

essential metabolites and transporting the vitamin A derivative, retinal. Furthermore, the RPE is essential for the phagocytosis of membranes shed by the photoreceptors and for photoreceptor renewal.

The thinned RPE layer in both groups of patients with PDR demonstrates disruption of normal RPE-photoreceptor complex anatomy, structural findings that correlate well with the compromised RPE and photoreceptor function in these patients, as measured by photostress recovery and dark adaptation. The additional presence of IS/OS thinning following PRP treatment may reflect further impairment in RPE-photoreceptor complex function and perhaps a reduced capacity for photoreceptor renewal following laser treatment. To our knowledge, the finding of thinned RPE in treatment-naïve patients with PDR has not been reported previously. It should be noted, however, that the axial resolution of the Spectralis SD-OCT is approximately 7 μm , and the global mean RPE thicknesses ranged from 25.6 μm among Post-PRP PDR patients to 29.0 μm among control patients in this study. Further investigation is still needed to validate the ability of the Spectralis SD-OCT to detect such subtle changes in RPE structure. However, previous studies have provided functional and anatomical evidence for pathological changes in the RPE-photoreceptor complex during diabetes. Degeneration of the RPE has been described in streptozotocin-induced diabetic rats, and depletion of occludin and loss of tight junction integrity was demonstrated in diabetic mice.⁴⁷⁻⁴⁹ In humans, postmortem diabetic eyes have localized extravascular albumin at sites of blood-retinal-barrier compromise.⁵⁰ Bek examined areas of capillary occlusion in 12 donor eyes with diabetic retinopathy and revealed, in 21 of the 27 studied lesions, an eosinophilic substance between the photoreceptor outer segments and the RPE.⁵¹ More recently, Decanini et al. analyzed the RPE proteome in pre-retinopathic diabetic donor eyes and in age-matched control donors.⁵² They found reduced expression of enzymes that regulate energy, protein and retinoid metabolism, and chaperone proteins that modify cell survival. Sixty-two percent of the RPE proteins altered in diabetic donor eyes also change in non-retinal tissues in diabetes, indicating that the RPE is compromised as part of the systemic impact of diabetes.⁵² Significant reductions in central choroidal thickness have also been demonstrated in individuals with diabetic retinopathy compared to controls using enhanced-depth imaging OCT⁵³. The choroid plays an essential role in nourishing the RPE and outer retina so thinning of the choroid is likely related to retinal tissue metabolic activity. The SD-OCT data from this study augments the current body of evidence for RPE degeneration in diabetes by demonstrating *in vivo* the presence of RPE-photoreceptor complex pathology in treated and untreated patients with PDR.

In conclusion, all patients with PDR exhibited inner retinal dysfunction and changes in inner retinal structure compared with controls. These results enhance an existing understanding of the nature and magnitude of retinal neurodegeneration in advanced stages of diabetic retinopathy. In particular, these findings draw attention to the compromised outer retinal structure and function in both untreated and treated patients with PDR. Detailed analysis of changes in outer retinal function and structure in patients with advanced diabetic retinopathy may guide the development of restorative vision therapies for patients with PDR.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Percent of participants falling outside reference range for each visual function test. Normal reference range for each test was defined by the mean performance of the control group $\pm$ 2 SD. *Presented in brackets are the p-values comparing the mean performances of the Post-PRP PDR and Treatment-naïve PDR groups on each visual function test, using independent t-tests or Mann-Whitney U tests as appropriate. FDP: frequency doubling perimetry; MD: mean deviation; HFA: Humphrey Field Analyzer.

Figure 2.

Displayed are the fundus photos and visual function test results of two Post-PRP patients. These patients had similar visual acuities and both received dense PRP. However, Patient One demonstrated normal photostress recovery time and dark adaptation speed, while Patient Two had significant impairments in both of these tests. Patient Two reported difficulty driving, poor peripheral vision, and poor vision in both dim and extreme lighting conditions.

Figure 3.

Graphs showing retinal layer thicknesses as semi-automatically segmented from SD-OCT scans in 9 ETDRS areas in the macula. Mean results for Post-PRP PDR subjects (PT), Treatment-naive PDR subjects (TN), and Controls (C) are shown. (a) RNFL: retinal nerve fiber layer; (b) GCL + IPL: ganglion cell layer + inner plexiform layer; (c) INL: inner nuclear layer; (d) OPL + ONL: outer plexiform layer + outer nuclear layer; (e) IS/OS: inner segment outer segment layer; (f) RPE: retinal pigment epithelium. An asterisk (*) indicates a significant difference in mean thickness between at least two patient groups, as indicated.

Table 1

Subject Characteristics

	Control	Treatment-naïve PDR	Post-PRP PDR	p-value [*]	Post-hoc p-value to compare PDR groups ^{**}
Patients, number	15	15	30		
Mean age, yrs (SD)	56.2 (17.7)	48.4 (15.9)	58.7 (13.5)	.106	.027
Mean diabetes duration, yrs (SD)	n/a	25.4 (12.9)	37.4 (12.8)	n/a	.005
Mean HbA1c% (SD)	5.6 (0.3)	8.9 (1.9)	7.4 (1.1)	<.0001	.003
Visual acuity, EVA score (SD)	90.1 (4.5)	79.3 (9.4)	78.5 (8.8)	<.0001	.759
Mean Spherical Equivalent (SD)	−.6 (1.9)	−1.4 (1.7)	0.0 (1.2)	.027	.012
IOP, mmHg (SD)	15.2 (3.6)	16.6 (4.1)	15.7 (2.8)	.514	.390

* For three group comparisons, ANOVA or Kruskal-Wallis tests were performed as appropriate.

** For post-hoc comparisons between Post-PRP PDR and Treatment-naïve PDR groups, independent t-tests or Mann-Whitney U tests were performed as appropriate. SD: standard deviation; IOP: intraocular pressure.

Table 2

Performance on Visual Function Tests

	Control	Treatment-naïve PDR	Post-PRP PDR	p-value [*]	p-value comparing PDR groups ^{**}	Age adjusted p-value comparing PDR groups ^{***}
Contrast Sensitivity (log) Mean (SD)	1.80 (0.14)	1.56 (0.20)	1.42 (0.17)	<0.0001	0.024	0.032
Dark Adaptation (minutes) Mean (SD)	9.74 (2.56)	10.22 (1.99)	12.80 (5.15)	0.052	0.144	0.387
FDP: fovea (decibels) Mean (SD)	30.00 (5.09)	22.60 (5.99)	22.07 (7.38)	<0.0001	0.763	0.564
FDP: MD (decibels) Mean (SD)	1.07 (2.50)	-5.48 (4.48)	-8.20 (5.76)	<0.0001	0.118	0.077
Reading acuity (logMAR) Mean (SD)	-0.01 (0.06)	0.16 (0.13)	0.23 (0.19)	<0.0001	0.191	0.451
HFA: 10-2 MD (decibels) Mean (SD)	0.05 (0.72)	-3.21 (2.86)	-4.52 (3.20)	<0.0001	0.185	0.154
HFA: 60-4 total threshold (decibels) Mean (SD)	1177.40 (139.07)	803.20 (277.08)	316.86 (281.44)	<0.0001	<0.0001	<0.0001
Photostress recovery time (seconds) Mean (SD)	70.64 (47.14)	93.21 (74.19)	151.02 (104.43)	0.020	0.060	0.093

SD: standard deviation; FDP: frequency doubling perimetry; HFA: Humphrey Field Analyzer.

* For three group comparisons, ANOVA or Kruskal-Wallis tests were performed as appropriate.

** For post-hoc comparisons between Post-PRP PDR and Treatment-naïve PDR groups, independent t-tests or Mann-Whitney U tests were performed as appropriate.

*** A nonparametric analysis of covariance model, adjusting for age as a covariate, was also used for comparisons between Post-PRP PDR and Treatment-naïve PDR groups.

Table 4

Global Mean Retinal Layer Thicknesses in Patients with PDR and Control Subjects

Retinal Layer	Global Mean Layer Thickness, μm (SD)		p-value*	p-value comparing PDR groups		p-value comparing Post-PRP PDR and control groups		p-value comparing Treatment-naïve PDR and control groups	
	Control	Treatment-naïve PDR		Control	Post-PRP PDR	Control	Post-PRP PDR	Control	Post-PRP PDR
NFL	38.09 (3.63)	41.67 (7.20)	48.41 (9.76)	<0.0001	0.026	0.024	<0.0001	<0.0001	0.110
GCL+IPL	70.77 (6.01)	69.05 (10.61)	67.84 (8.25)	0.544	0.682	0.877	0.229	0.280	0.591
INL	32.47 (2.52)	31.60 (4.26)	31.85 (5.21)	0.859	0.875	0.709	0.594	0.597	0.505
OPL+ONL	101.93 (5.50)	114.97 (13.12)	105.41 (13.46)	0.012	0.033	0.015	0.227	0.384	0.003
IS/OS	33.31 (2.52)	31.69 (2.40)	29.35 (5.33)	0.012	0.052	0.196	0.002	0.011	0.087
RPE	29.03 (4.09)	25.92 (3.14)	25.64 (2.81)	0.005	0.763	0.810	0.009	0.003	0.031
									0.013

SD: standard deviation; global: global average layer thickness; NFL: nerve fiber layer; GCL+IPL: ganglion cell layer + inner plexiform layer; INL: inner nuclear layer; OPL+ONL: outer plexiform layer + outer nuclear layer; IS/OS: inner segment/ outer segment photoreceptor layer; RPE: retinal pigment epithelium.

* For three group comparisons, ANOVA tests were performed.

** For post-hoc comparisons between two groups, independent samples t-tests were performed.

*** Analysis of covariance, adjusting for age, was also used for comparisons between two groups.